An Unusual Case of Philadelphia Chromosome–Positive Chronic Myelogenous Leukemia With Trisomy 19 Presenting With Megakaryoblastosis and Myelofibrosis

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- Initial identification of chronic myelogenous leukemia is very important since targeted therapy leads to life-saving remission. Rarely, chronic myelogenous leukemia presents with an unusual picture, making the diagnosis challenging. We describe such a case of chronic myelogenous leukemia in blast crisis in a previously healthy 61-year-old woman. The patient presented with fever, myalgias, and night sweats and was first worked up for an infectious etiology. Because of persistent anemia, a bone marrow biopsy was performed that revealed fibrosis with increased megakaryoblasts. Even though initial cytogenetic studies could not be performed because of “dry tap” aspirate, persistent efforts for cytogenetic studies were made, including a “squeeze preparation” from the core biopsy, which revealed t(9;22)(q34;q11.2) and trisomy 19. The patient was treated with tyrosine kinase inhibitors, chemotherapy, and subsequently an allogeneic stem cell transplant. She is in persistent remission. This case illustrates a complex presentation of chronic myelogenous leukemia and provides an overview of morphologic cues and the importance of performing cytogenetic studies that led to the diagnosis.


Accurate diagnosis of chronic myelogenous leukemia (CML) is important because targeted therapy with tyrosine kinase inhibitors (TKIs) can be instituted, which induces life-saving remission in a majority of patients. Chronic myelogenous leukemia is diagnosed by demonstrating chromosomal translocation involving the BCR-ABL genes: t(9;22). Typical presentations of CML include high white count dominated by mature myeloid cells, with basophilia and eosinophilia in the peripheral blood, and a bone marrow showing a myeloid dominant maturation with atypical micromegakaryocytes in tight clusters. The abnormal complete blood count parameters and atypical morphology trigger appropriate tests (fluorescence in situ hybridization or molecular based) to confirm the presence of BCR-ABL translocation, which is essential for diagnosing CML. Rarely, patients with CML have unusual presentations and a suspicion of CML may not be entertained by the diagnosing pathologist, especially when the disease does not fulfill the clinical and morphologic criteria needed to trigger confirmatory cytogenetic/molecular studies for CML.

Chronic myelogenous leukemia with additional karyotypic abnormalities may have an unusual presentation and a more aggressive course similar to that of acute leukemia. There have been a few case reports published of Philadelphia chromosome–positive acute myeloid leukemia (Ph+ AML) presenting as megakaryoblastic blast crisis. In addition, there have been rare reports of comparisons of acute megakaryoblastic leukemia and CML with trisomy 19 presenting with blast crisis. Acute megakaryoblastic leukemia may present as panmyelosis with myelofibrosis, usually considered in the differential diagnosis of either entity.

We report an unusual case of atypical presentation of CML with trisomy 19 presenting as panmyelosis with myelofibrosis, with predominance of atypical megakaryocytes, in a 61-year-old, previously healthy woman. The clinical and morphologic features usually did not fit into the criteria for a diagnosis of CML or atypical CML, as defined by the World Health Organization classification of tumors of hematopoietic and lymphoid tissues. This article will highlight the unique presentation, morphologic features, and bone marrow extraction techniques that were helpful in establishing the diagnosis and management of this patient.

CASE PRESENTATION

A 61-year-old, previously healthy woman presented to the emergency department with a 2-week history of fevers, fatigue, myalgias, rigors, and night sweats. She also had a nonproductive cough and postnasal drip. She had visited her primary care physician, who started her on antibiotics for presumed sinus infection and possible Lyme disease because of a history of her dogs having tick bites and a diagnosis of Lyme disease. After a few days of antibiotics, her myalgias and night sweats resolved but the fevers and fatigue continued. Her primary care physician received her laboratory results, which revealed a low hematocrit of 21.8%.
(reference range, 36%–48%), and referred her to the emergency department for further evaluation of persistent fevers and anemia. On review of her hemogram, she also had low hemoglobin of 7.2 g/dL (reference range, 12.0–16.0 g/dL). However, the white blood cell count was 7 600/µL (reference range, 4 000–11 000/µL), with a relatively normal differential count of 65% neutrophils, 20% lymphocytes, 3% monocytes, and moderately increased basophils at 6%. The platelet count was normal at 2.64 × 10^6/µL (reference range, 1.50–4.40 × 10^6/µL) and lactose dehydrogenase level on admission was high at 544 U/L (reference range, 94–250 U/L). Physical examination was significant for a palpable spleen (1–2 cm below the left costal margin), which on computed tomographic imaging measured 15 cm in craniocaudal dimension.

The patient was extensively worked up for an infectious etiology of her fevers and anemia and, although the studies were negative for viral or bacterial etiologies, her peripheral blood smear showed a leukoerythroblastic picture with occasional circulating myeloid precursors, nucleated red blood cells, and teardrop erythrocytes. A bone marrow biopsy was performed; the aspirate was unsuccessful (“dry tap”) but the biopsy material showed a fibrotic bone marrow with atypical megakaryocytes and megakaryoblasts (Figure 1, a). The dominant marrow finding was extensive marrow (collagen) fibrosis grade 1/4 (Figure 1, b) along with sheets of highly atypical megakaryocytes and megakaryoblasts. Focal areas with eosinophils were present, with increased myelopoiesis (Figure 1, a). Myeloblasts in the bone marrow core biopsy, by hematoxylin–eosin morphology or by CD34 immunohistochemical stains, were approximated at 5%. However, the megakaryocytes and megakaryoblasts highlighted by CD42 immunohistochemical stain (1:100; clone MM2/174, Novocastra, Buffalo Grove, Illinois; Figure 2), comprised approximately 60% of marrow cellularity. The marrow findings, in conjunction with clinical findings, raised a few differential diagnostic possibilities: primary myelofibrosis, acute megakaryoblastic leukemia, or acute panmyelosis with myelofibrosis. The rapid clinical evolution favored the latter two. Because focal areas of myeloid cells and eosinophils raised the rare possibility of CML, cytogenetic studies were also pursued despite the dry tap. Meanwhile, a repeat bone marrow specimen was obtained in order to establish a definitive diagnosis. Because this was also a dry tap, a squeeze (explained in “Comment”) preparation was attempted from the fibrotic core, in order to establish cells for cytogenetic and fluorescence in situ hybridization studies to supplement studies from her peripheral blood. Chromosomal analysis revealed a t(9;22)(q34;q11.2) in 19 of 20 metaphases plus a trisomy 19 in 14 of 19 metaphases with t(9;22) (Figure 3, a). Fluorescence in situ hybridization evaluation for a BCR-ABL1 rearrangement was performed (with Vysis LSI BCR/ ABL1 Dual Color, Dual Fusion Translocation Probe, catalog number 0582-001, Abbot Vysis Molecular, Abbot Park, Illinois) and the rearrangement was seen in 95 of 100 nuclei (Figure 3, b).

The presence of trisomy 19 as a new finding in a patient with a known diagnosis of CML is usually seen in patients with accelerated or blast crisis of CML. The diagnostic criteria for the presence of trisomy 19 along with t(9;22) at the time of presentation are not clearly defined by the present World Health Organization classification. However, the rapid progression in clinical course in a relatively short time, along with the presence of increased megakaryoblasts

Figure 1. a, Bone marrow biopsy with extensive fibrosis (bottom) and clusters of atypical megakaryocytes and megakaryoblasts (middle), admixed with focal areas of myelopoesis and abundant eosinophils (top). b, Bone marrow biopsy with extensive marrow collagen fibrosis, highlighted by trichrome stains. Grade 4/4; myelofibrosis 2–3 (hematoxylin–eosin, original magnification ×200 [a]; original magnification ×200 [b]).

Figure 2. CD42 highlights an increase in megakaryocytes and megakaryoblasts in small clusters (immunoperoxidase, original magnification ×1000).
in bone marrow, prompted a clinical consideration for blast crisis phase of CML, and the patient was managed as evolving into an acute leukemia. She was started on induction chemotherapy with cytarabine and daunorubicin. The TKI imatinib was added to her chemotherapy regimen subsequently. After a phase of initial recovery (week 3), the patient suffered adverse effects of chemotherapy, including infections and cytopenias (probably related to TKI), and did

**Figure 3.** a, Karyogram showing t(9;22)(q34;q11.2) with concurrent trisomy 19. b, Fluorescence in situ hybridization performed on nuclei with BCR/ABL1 Dual Color, Dual Fusion Translocation Probe (Abbott Vysis Molecular, Abbot Park, Illinois), showing the abnormal rearrangement leading to t(9;22)(q34;q11.2).

**Figure 4.** Bone marrow at follow-up (6 months) shows normal hematopoiesis. Cytogenetic and molecular studies were negative for the presence of t(9;22) or BCR-ABL transcripts (hematoxylin–eosin, original magnification x200).
not recover her complete blood count. Because she remained pancytopenic, requiring frequent blood transfusions, an allogeneic stem cell transplantation (6/6 matched related donor) was performed a month after her initial presentation. About 6 months after her transplant, the patient was in morphologic, cytogenetic, and molecular remission from her CML with trisomy 19. The follow-up bone marrow photomicrograph after 6 months is shown in Figure 4.

**COMMENT**

A diagnosis of CML is usually straightforward for the pathologist and hemat-oncologist and is a diagnosis associated with a positive outcome for the patient, because a majority of these patients are treated successfully to disease remission with TKIs. Thus, since the inception of TKI therapy around the turn of this century, identification of CML and its variants are very important for patient management. In a minority of cases, diagnosing CML is a challenge, especially when CML presents as a transformed (accelerated or blast-phase) disease.

In our case, the predominance of fibrosis and megakaryocytes at presentation confounded the initial diagnosis. Although very uncommon, such presentations in CML have been reported before. In an original observational study among 103 patients with CML, correlating cytogenetics to bone marrow histopathology, the authors described 4 separate kinds of CML: common type, overlapping, megakaryocyte increase, and megakaryocyte predominance. Additional karyotypic abnormalities were detected in 8 of the 45 cases of the common type and 12 of the 42 cases with the latter 2 types. Myelosclerosis and myelofibrosis were seen in many cases associated with marrow megakaryocytosis. Of the 4 cases (2 accelerated phase and 2 blast phase) they reported with transformed disease, 2 of the cases with excess blasts (accelerated phase) had increased megakaryocytes. They elegantly describe 3 of the 20 patients with additional chromosome 19. Of these 3 cases, 1 was isolated trisomy 19 (in addition to BCR-ABL translocation) and the other 2 showed a complex karyotype.

However, when a patient presents without leukocytosis, and the marrow does not show increase in myeloid precursors, CML is usually never in the main differential diagnostic consideration. In addition, when there is leukoerythroblastosis in the peripheral blood along with increased fibrosis and increased atypical megakaryocytes and megakaryoblasts, then the differential diagnosis includes acute megakaryoblastic leukemia, acute panmyelosis with myelofibrosis, and transformed primary myelofibrosis.

Acute megakaryoblastic leukemia, according to the World Health Organization classification,1 is defined as an "acute leukemia with 20% or more blasts of which at least 50% are of megakaryocyte lineage." It constitutes less than 5% of all acute myeloid leukemia cases and usually presents with cytopenias without hepatosplenomegaly. There are a few different chromosomal abnormalities seen in this condition, such as trisomy 8, trisomy 19, monosomy 7, and abnormalities of the long arm of chromosome 3, among others, none of which are specific for this type of leukemia.4,9

Acute megakaryoblastic leukemia is commonly associated with Down syndrome and with only one other chromosomal abnormality, consistently seen in approximately one-third of pediatric patients, t(1;22)(p13;q13).4,10 In the differential diagnosis for our case we entertained the possibility of acute panmyelosis with myelofibrosis, and megakaryoblastic crisis of any myeloproliferative neoplasm, including transformed CML. Megakaryoblastic leukemia has overlapping features with acute panmyelosis with myelofibrosis and, as seen in this case, these entities are sometimes difficult to distinguish from each other. Acute panmyelosis with myelofibrosis usually presents with pancytopenia with extensive fibrosis and increased blasts of erythroid, myeloid, and megakaryocytic lineages seen on bone marrow biopsy; there is no specific cytogenetic abnormality associated with this entity.1 CML in blast crisis is usually associated with a history of a chronic phase with basophilia and splenomegaly, as well as the BCR-ABL fusion gene. Blast-phase CML is typically a result of clonal evolution from chronic phase CML. The blast-phase transformation is indicative of an aggressive phase of the disease,3,6 and is managed as for a patient with acute leukemia. The majority of CML cases that progress to blast crisis may acquire additional chromosomal abnormalities, in addition to the BCR-ABL, such as extra copies of the Ph chromosome, trisomy 8 and 19, and i(17)(q10).3,6 Interestingly, trisomy 19 has been reported as a frequently gained mutation in cases of acute megakaryoblastic leukemia in adults.4,13

The prognostic significance of trisomy 19 is not clearly known. A 2004 review92 shows that trisomy 19 occurs in about 13% of CML patients with blast-phase disease. Trisomy 19 is thought to be acquired in the late phase of the disease. It is not known whether BCR-ABL has a direct role in promoting genetic instability inducing random duplications and trisomies in immature stem cells or if specific genes in chromosome 19 can contribute to progression of disease. None of the 4 known abnormally mutated genes in blast phase of CML (p53 [chromosome 17], p16/ARF [chromosome 9], Rb [chromosome 13], and RAS [chromosome 12]) are localized to chromosome 19.

The clinical and morphologic features that prompted us to consider an uncommon evolution from a myeloproliferative neoplasm were splenomegaly and pockets of bone marrow eosinophils. In addition, many small megakaryoblasts were seen, some indistinguishable from micromegakaryocytes typically seen in CML (Figure 1, a).

Many other previous studies have shown similar challenges, where the differential diagnosis was between acute megakaryocytic leukemia and CML in leukemic evolution. Soupir et al3 performed a multi-institutional retrospective analysis of cases of Ph+ AMLs and CML in blast crisis and showed that Ph+ AMLs differed from CML in blast crisis by different clinical pictures (no splenomegaly nor basophilia in Ph+ AML), bone marrow morphology (lower cellularity and myeloid to erythroid ratio in Ph+ AML), cytogenetic findings (characteristic other chromosomal abnormalities usually found in CML were not seen in Ph+ AML), and response to chemotherapy (return to normal karyotype was observed in cases of Ph+ AML, whereas persistence of Ph+ was seen in CML); however, the median survival was similar between the 2 groups. A case report by Pelloso et al8 of a 25-year-old woman who presented with findings suggestive of leukemia raised a differential diagnosis of acute megakaryocytic leukemia with t(9;22)(q34;11) and megakaryoblast crisis as first-time presentation of CML. Pelloso et al8 used clinical, morphologic, and cytogenetic findings to classify this case as more likely to be CML that presented in megakaryocytic blast crisis; however, they
noted that there is much dispute in this distinction. Similarly, a case reported by Wu et al\textsuperscript{13} of a 38-year-old man was thought to represent CML presenting in megakaryoblast crisis because of the presence of a t(9;22)(q34;q11), peripheral leukocytosis with basophilia and a response to treatment more often seen in CML patients. A study by Pullarkat et al\textsuperscript{6} argued that their ability to distinguish by cytogenetic studies which cells (blasts versus neutrophils) had aberrant BCR-ABL fusion and how many of the copies of these chromosomal abnormalities were present in these cells was helpful in determining the difference between Ph\textsuperscript{+} acute megakaryoblastic leukemia and megakaryoblast crisis of CML.

The persistence in our attempts to obtain cytogenetic studies from a dry aspirate was also a key step in establishing a diagnosis. The protocol used to isolate cells from bone marrow core biopsies from a dry tap was initially described by one of our colleagues.\textsuperscript{7} The protocol uses a fresh fragment of a core biopsy (unfixed), or alternatively a 0.2-cm-long fragment separated from the terminal deep end of the core biopsy obtained for histology. The fragment is mechanically disaggregated using a blunt forceps. The fragment is held under saline, and a blunt forceps is used to squeeze/tweeze the tissue, expressing marrow cells into the saline suspension, under which the core fragment is held. This method yields cell suspensions from a dry-tap bone marrow core biopsy that may be used for Giemsa staining, flow cytometry, and cytogenetics.

The case presented in this manuscript had several challenging features. The clinical and morphologic features at the outset did not fit a diagnosis of CML, chronic phase, accelerated phase, or blast crisis, as defined by the World Health Organization blue book\textsuperscript{1}. However, the unusual presentation, along with pockets of eosinophils noted in the marrow core biopsy, prompted us to consider this remote diagnosis of a myeloproliferative neoplasm. Additionally, a rigorous pursuit to obtain karyotype from repeated dry-tap aspirates, including an uncommonly performed maneuver (squeeze preparation from the core biopsy), led us to finally identify Philadelphia chromosome in addition to trisomy 19. These challenging features along with persistence in pursuing a cytogenetic diagnosis are valuable lessons learned from this interesting case.

References